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Crystallographic Investigations on the Structure-Function-Relationship in Tryptophan Synthase

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Introduction: Tryptophan synthase (TRPS) is a tetrameric bifunctional enzyme that catalyses the last two steps in the biosynthesis of tryptophan. Its alpha- and beta-subunits are arranged in a linear alpha-beta-beta-alpha fashion with the active centers of the alpha- and beta-subunits interconnected by a 25 Å long tunnel [1]. The alpha-subunit catalyses the cleavage of indole-glycerole-phosphate (IGP) to indole and glyceraldehyde-3-phosphate (G3P) (alpha-reaction), in the beta-subunit indole is condensed with serine to tryptophan and water in a pyridoxal-5'-phosphate (PLP) dependent reaction (beta-reaction). The common reactant indole diffuses from the alpha- to the beta-active site through the tunnel thereby preventing its loss through the cell membranes. In order to keep the two reactions in phase, the alpha- and beta-reactions regulate each other reciprocally by allosteric interactions. An important feature is the shuttling between open (high-activity) and closed (low-activity) conformations, with the equilibrium depending on the ligands bound to the alpha-active site and the covalent intermediate formed at the beta-active site. Upon serine binding, the internal aldimine (E(Ain)) between PLP betaLys87 is replaced by the external aldimine ($E(Aex_1)$) with serine. Subsequent formation of an aminoacrylate intermediate (E(A-A)) triggers an isomerization of the alpha-active site to a high-activity closed form. Indole is cleaved off at the alpha-active site, channeled to the beta-active site where it reacts with the aminoacrylate to form first a quinoinoid($E(Q_3)$), and then an aldimine intermediate ($E(Aex_2)$), ultimately yielding tryptophan. Formation of $E(Aex_2)$ returns the alpha-active site to the low activity form. Other important parameters in the allosteric interactions between the alpha- and beta-subunits are pH, temperature, and monovalent cations (MVC). In order to obtain a more detailed insight in the beta-reaction we determined the structure of the quinonoid intermediate ($E(Q_3)$) of the wildtype enzyme. In addition, we analyzed the function of the highly conserved betaGln114 by determining the structures of the native and serine complexed mutant betaGln114Asn.

Methods and Materials:Wildtype and mutant TRPS was purified and crystallized in the monoclinic spacegroup C2 (a=184, b=60.0, c=67.5, beta=94.6) as described previously [2]. The quinonoid complex was generated by soaking the crystals in ceasium chloride, glyceraldehyde-3-phosphate, and indoline [3]. The crystals were cryo-cooled in liquid nitrogen.

Results: Two datasets were collected at 100 K to 1.9 and 2.0 A resolution of two slightly differently prepared quinonoid complexes. Initial refinement indicates that both contain the quinonoid at the beta active site. Refinement is still in progress. Two datasets were collected at 100 K to 1.7 A resolution of the betaGln114Asn mutant, in its native and serine complexed form. Serine is clearly visible at the beta active site forming an external aldimine. betaAsn114 forms a hydrogen bond with the O3 oxygen of the pyridoxal phosphate cofactor. Refinement of the structures is in progress.

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